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Novel Loci Associated with Increased Risk of Sudden Cardiac Death in the Context of Coronary Artery Disease

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Abstract

Background: Recent genome-wide association studies (GWAS) have identified novel loci associated with sudden cardiac death (SCD). Despite this progress, identified DNA variants account for a relatively small portion of overall SCD risk, suggesting that additional loci contributing to SCD susceptibility await discovery. The objective of this study was to identify novel DNA variation associated with SCD in the context of coronary artery disease (CAD).

Methods and Findings: Using the MetaboChip custom array we conducted a case-control association analysis of 119,117 SNPs in 948 SCD cases (with underlying CAD) from the Oregon Sudden Unexpected Death Study (Oregon-SUDS) and 3,050 controls with CAD from the Wellcome Trust Case-Control Consortium (WTCCC). Two newly identified loci were significantly associated with increased risk of SCD after correction for multiple comparisons at: rs6730157 in the *RAB3GAP1* gene on chromosome 2 ($P = 4.93 \times 10^{-12}$, OR = 1.60) and rs2077316 in the *ZNF365* gene on chromosome 10 ($P = 3.64 \times 10^{-8}$, OR = 2.41).

Conclusions: Our findings suggest that *RAB3GAP1* and *ZNF365* are relevant candidate genes for SCD and will contribute to the mechanistic understanding of SCD susceptibility.

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† The full list of WTCCC+ members is provided in File S1.

‡ NJS and SSC are joint senior authors on this work.

Introduction

Sudden cardiac death (SCD) remains a significant public health problem with an estimated annual incidence of 250,000–300,000 in the US and 4–5 million around the globe [1–3]. Although coronary artery disease (CAD) underlies the majority of SCD [4], there is a significant familial component to SCD risk which appears to be distinct from that associated with other manifesta-

tions of atherosclerosis in population-based studies [5–7]. Recent collaborative genome-wide association (GWA) efforts have identified susceptibility loci associated with SCD [8–10] but only two DNA variants on chromosomes 2q24 (*BAZ2B*) [10] and 21q21 (near *CXADR*) [9] have crossed the stringent threshold of genome-wide statistical significance. While candidate-gene based studies have also yielded DNA variants associated with SCD these may not constitute an unbiased approach [11–13]. We hypothesized

that the distinct configuration of the MetaboChip custom array which contains variants nominally associated ($P < 0.01$) with CAD, QT interval, systolic and diastolic blood pressure, diabetes, glycemic traits, lipids, height and weight in large-scale meta-analyses of GWA studies [14] would enable the identification of additional novel genetic variation associated with SCD in the context of CAD. Accordingly, we conducted a case-control association study using SCD cases from the Oregon Sudden Unexpected Death Study (Oregon-SUDS) and controls with CAD from the Wellcome Trust Case-Control Consortium (WTCCC+).

Methods

Study Subjects

Ethics statement. All samples have been established in accordance with the principles expressed in the Declaration of Helsinki. The study was approved by the Institutional Review Boards of Oregon Health and Science University, Legacy Health Systems, VA Medical Center, Portland OR and the WTCCC Data Access Committee. Written informed consent was obtained from all enrolled subjects. If subjects were deceased at the time of ascertainment (i.e. following a sudden cardiac death) consent was waived by the respective Institutional Review Boards on grounds of scientific feasibility. These latter subjects were de-identified for the purpose of analysis, in conformation with procedures approved by the respective Institutional Review Boards.

Oregon-SUDS. A total of 979 SCD cases of European descent were ascertained from the Oregon-SUDS, an ongoing community-based study among residents of the Portland, Oregon metropolitan area. Detailed methodology has been reported previously [15–18]. Briefly, SCD cases are identified from the emergency medical response system, the medical examiner network and 16 local hospitals. All available medical records are obtained for each subject. SCD was defined as an unexpected pulseless condition likely of cardiac origin. If unwitnessed, SCD was defined as unexpected death within 24 hours of having last been seen alive and in normal state of health [19]. All SCD cases included in this study were required to have documented coronary artery disease (CAD). CAD was defined as 50% stenosis of a major coronary artery, physician report of past myocardial infarction (MI), history of percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG); or autopsy-identified CAD; or MI by clinical data with any two of the following three: ischemic symptoms, positive troponins or CKMB; or pathologic Q waves on ECG. SCD cases with chronic terminal illnesses, known non-cardiac causes of SCD, traumatic deaths and drug overdose were excluded from the analysis.

WTCCC+ controls. The control samples for this study comprised 3,219 pooled subjects from 4 UK studies: (i) CAD cases recruited into the British Heart Foundation Family Heart Study [20] ($n = 2169$, 78.6% males, 70.8% MI, 68.6% CABG/PCI, mean age at diagnosis 49.9 ± 7.7 years), including the 1,926 subjects analyzed in the original WTCCC Study [21]; (ii) Young MI cases with an event below the age of 50 years recruited into the Premature Acute Myocardial Infarction Study (PRAMIS) [22] ($n = 214$, 85.5% males, mean age at event 42.4 ± 5.8 years); (iii) MI cases recruited into the Secondary Prevention of Acute Coronary Events - Reduction of Cholesterol to Key European Targets (SPACE ROCKET) Trial [23] ($n = 499$, 84.0% males, mean age at event 57.7 ± 8.4 years); (iv) MI cases recruited in the Outcomes from Percutaneous coronary intervention by Evaluation of Risk Attributes (OPERA) Trial [24] ($n = 337$, 75.5% males, mean age at event 55.8 ± 8.4 years). CAD status and diagnosis was validated in all studies by direct review of clinical notes. All subjects were of

White European origin. The choice of CAD controls (as opposed to population-based controls) was based on the recognition that over 80% of SCDs occur in the setting of CAD. Accordingly, CAD controls would enable the discovery of genetic associations exclusively related to SCD and independent of CAD [25].

MetaboChip Array

The MetaboChip array (Illumina, San Diego, CA) is a custom Illumina iSelect genotyping array comprised of approximately 200,000 SNPs selected from previous GWAS meta-analyses findings from the CARDIoGRAM (coronary artery disease), DIAGRAM (type 2 diabetes), GIANT (height and weight), MAGIC (glycemic traits), Lipids (lipids), ICBP-GWAS (blood pressure), and QT-IGC (QT interval) consortia [14]. The array comprises a linkage-disequilibrium pruned set of SNPs that reached a nominal level ($P < 0.01$) of association with each of these diseases/phenotypes as well as SNP sets for fine-mapping of loci identified for these disease/traits at the time of design of the array. Additional details of the MetaboChip design can be found at: www.sph.umich.edu/csg/kang/MetaboChip/.

MetaboChip Genotyping and Statistical Analysis

Genotyping of SCD cases was performed at the Medical Genetics Institute at Cedars-Sinai Medical Center, Los Angeles, U.S. and controls were genotyped at the Wellcome Trust Sanger Centre, Hinxton, UK using the same array. After exclusion of array failures, poor quality genotypes and duplicates, 948 SCD cases and 3,050 CAD controls were used in the current analyses.

Genotypes in both studies were called using the GenCall algorithm [26,27]. Individual SNPs were excluded from analysis using standard quality control criteria based on sample call rates less than 90%, Hardy-Weinberg Equilibrium (HWE) $P < 1 \times 10^{-4}$, monomorphic and SNPs with minor allele frequencies (MAF) less than 1%. This left 119,117 post-QC SNPs for analysis. Association analyses were performed using logistic regression assuming an additive model adjusting for age, sex and the first 3 dimensions from multi-dimensional scaling (PLINK software) [28]. Results were further corrected for the genomic control factor (λ), which was calculated after excluding SNPs related to QT interval and CAD.

Results

The mean ages of the subjects in the Oregon-SUDS and the WTCCC studies were 60.8 ± 12.6 and 51.4 ± 7.5 years respectively at time of event. Seventy two percent of SCD cases and 80.9% of CAD controls were male. The genomic control factor (λ) for this analysis was 1.25. Based on the number of SNPs tested (119,117 SNPs) a significance P -value cut off of 4.2×10^{-7} was determined. This level of correction for multiple testing is probably conservative given that the MetaboChip array contains many DNA variants in strong linkage disequilibrium, especially within the fine-mapping sets. Nonetheless, we observed SNPs exceeding the array-wide significance threshold ($P = 4.2 \times 10^{-7}$) as well as genome-wide significance ($P < 5 \times 10^{-8}$) after correcting for lambda inflation in two loci on chromosomes: 2q21 and 10q21 (Figure 1). The association results of the lead variants at each of these loci are shown in Table 1. The strongest associations were observed for the intronic SNPs rs6730157 ($P = 4.93 \times 10^{-12}$, OR = 1.60, 95%) within *RAB3GAP1* and rs2077316 in the *ZNF365* gene ($P = 3.64 \times 10^{-8}$, OR = 2.41).

The association signal on 2q21 spans quite a large region with multiple SNPs showing a significant association (Figure 1). In conditional analysis, rs6730157 remained the most significant SNP

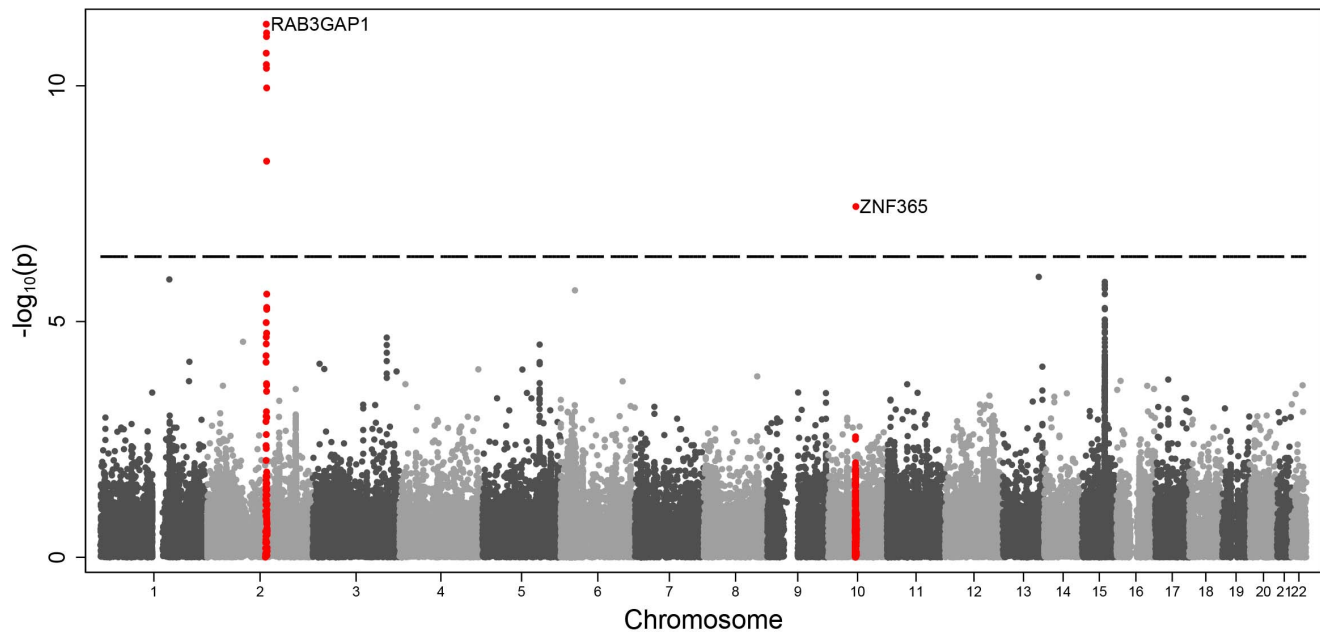


Figure 1. Manhattan plot of associated findings. Data is displayed as $-\log_{10}P$ values against chromosomal location for the 119,117 SNPs that were included in the statistical analysis. The dotted line represents the conservative significance threshold of $P = 4.2 \times 10^{-7}$. The two loci that showed an association at this level are plotted in red.
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and no other SNP in the region had a significant association ($P < 0.01$). Figure 2 shows the regional association plots for rs6730157 and rs2077316.

Discussion

We have conducted a case-control association study using the MetaboChip array to identify novel genetic variation associated with SCD independent of CAD. The most significantly associated SNP (rs6730157, $P = 4.93 \times 10^{-12}$) resides in an intronic region of the *RAB3GAP1* gene on chromosome 2q21. *RAB3GAP1* encodes the catalytic subunit of RabGTPase activating protein. *RAB3GAP*, which is involved in regulation of *RAB3* activity, is a heterodimeric complex consisting of a 130-kD catalytic subunit. Mutations in *RAB3GAP1* are associated with Warburg micro syndrome, a rare autosomal recessive syndrome characterized by microcephaly, severe mental retardation and cataracts [29]. *RAB3GAP1* is a key regulator of calcium mediated hormone and neurotransmitter exocytosis [30,31]. Interestingly, a previous study performed in a yeast two-hybrid system and a rat dorsal root ganglion found that a protein similar to human *RAB3GAP1* interacts with intracellular domains of *SCN10A* [32]. DNA variation within *SCN10A* has been associated with abnormalities of cardiac ventricular depolarization, conduction, and ventricular fibrillation [33–36].

To test whether rs6730157 was located in a regulatory region or transcription factor binding domain, we searched the ENCODE project (Encyclopedia of DNA elements) database. We found that rs6730157 is predicted to fall into a strong enhancer in several cell types, including cardiac and aortic adventitial fibroblast cells [37]. However, it should be noted that although *RAB3GAP1* is a strong candidate gene in the chromosome 2 locus, the association signal spans several other genes (Figure 2). At this stage, in common with other GWAS findings, we cannot exclude the possibility that the association is driven by another gene at this locus. Fine mapping and functional analysis of the locus will be required to refine the association.

The second significantly associated SNP (rs2077316, $P = 3.64 \times 10^{-8}$) resides in an intronic region of the zinc finger protein 365 gene (*ZNF365*) on chromosome 10q21. *ZNF365* encodes several isoforms which have different expression patterns and functions. *ZNF365* has been implicated in breast cancer [38] and Crohn's disease [39] and a role in heart disease has not been reported. According to ENCODE, no regulatory effects for rs2077316 are currently predicted [37].

Our study has several limitations. Despite attempting to take any population stratification into account using multi-dimensional scaling, we observed an inflation of the genomic control factor

Table 1. Summary of the two loci associated with SCD.

Chr	SNP	Gene	Position	Associated allele	Associated allele frequency SCD cases	Associated allele frequency CAD controls	SE	OR	λ Corrected P-value
2	rs6730157	<i>RAB3GAP1</i>	135623558	G	0.37	0.26	0.06	1.60	4.93×10^{-12}
10	rs2077316	<i>ZNF365</i>	63895454	C	0.060	0.026	0.14	2.41	3.64×10^{-8}

Chr, chromosome; SE, standard error; OR, odds ratio.
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Figure 2. Regional association plots for the two associated SNPs with SCD. Each SNP is plotted with respect to its chromosomal location (x-axis) and $-\log_{10}P$ -value (y-axis on the left). The spikes indicate the recombination rate (y-axis on the right) at that region of the chromosome. doi:10.1371/journal.pone.0059905.g002

statistic (λ). This could be due to further differences in population structure between the SCD cases and CAD controls which, while all of European descent, are drawn from individuals from two separate countries. Alternately, the design of the MetaboChip with a possible over-representation of variants of relevance given the choices of traits used to select the SNPs could contribute to an inflation of this statistic. We tried to limit the impact of this by excluding SNPs related to QT interval and CAD when calculating the genomic control factor statistic. Most importantly, our findings currently lack replication. In this context, although the association at the 2q21 locus looks robust (with the association exceeding GWA significance by several log values), particular caution needs to be exercised in the interpretation of the finding at 10q21 as only a single SNP with a very low minor allele frequency (Table 1) showed an association. Replication of the findings is challenging because of the rarity of collections of SCD subjects occurring in the context of CAD. Nonetheless, in both cases our findings should be considered provisional until further corroboration.

In summary, we provide evidence for two novel loci where variants may affect risk of SCD in the context of CAD. Understanding the mechanisms that increase risk of SCD is an

essential first step in trying to reduce this important complication of CAD.

Supporting Information

File S1 The full list of WTCCC+ members. (DOC)

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Author Contributions

Conceived and designed the experiments: AHV CPN NJS SSC. Performed the experiments: AHV CPN JA KJ MJ KDT. Analyzed the data: AHV CPN XG. Contributed reagents/materials/analysis tools: AHV CPN XG KR AUE CT JA KJ HC PSB PD ASH AJB MJ KDT SLP CNC KG JJ JIR CMA NJS SSC. Wrote the paper: AHV CPN NJS SSC.

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